Highly Monodisperse Water-Dispersable Iron Oxide Nanoparticles for Biomedical Applications

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Supporting Information

1. Experimental details

   a) The reaction schemes for the preparation of ligand (2) are shown in Figure 1-SI and Figure 2-SI.
Figure 1-SI. Synthesis of the key intermediate 7.

Figure 2-SI. Synthesis of the hydroxamic acid 10.

Synthesis of 11-(tert-butoxycarbonylamino)undecanoic acid (2): $^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ / ppm = 10.88 (br, 1H), 4.54 (br, 1H), 3.08 (m, 2H), 2.33 (t, $J = 7.5$ Hz, 2H), 1.65-
1.58 (m, 2H), 1.50-1.37 (m, 2H), 1.43 (s, 9H), 1.34-1.20 (m, 12H). $^{13}$C-NMR (100 MHz, CDCl$_3$): $\delta$/ ppm = 179.3 (s), 156.0 (s), 79.0 (s), 40.6 (t), 34.0 (t), 30.0 (t), 29.4 (t), 29.24 (t), 29.16 (t), 29.1 (t), 29.0 (t), 28.4 (q, 3C), 26.7 (t), 24.7 (t).

Synthesis of benzyl-11-(tert-butoxycarbonylamino)undecanoate (3): m.p. 31-32 °C. $^1$H-NMR (400 MHz, CDCl$_3$): $\delta$/ ppm = 7.36-7.32 (m, 5H), 5.11 (s, 2H), 4.53 (br, 1H), 3.11-3.06 (m, 2H), 2.34 (t, J = 7.5 Hz, 2H), 1.67-1.58 (m, 2H), 1.47-1.40 (m, 2H), 1.43 (s, 9H), 1.31-1.22 (m, 12H). $^{13}$C-NMR (100 MHz, CDCl$_3$): $\delta$/ ppm = 173.6 (s), 155.9 (s), 136.1 (s), 128.5 (d, 2C), 128.1 (d, 3C), 78.9 (s), 66.0 (t), 40.6 (t), 34.3 (t), 30.0 (t), 29.4 (t), 29.3 (t), 29.2 (t), 29.1 (t), 29.0 (t), 28.5 (q, 3C), 26.7 (t), 24.9 (t).

Synthesis of 11-[tert-butoxycarbonyl-[2-[2-(2-methoxyethoxy)ethoxy]ethyl]amino]-undecanoic acid (4): $^1$H-NMR (400 MHz, CDCl$_3$): $\delta$/ ppm = 10.63 (br, 1H), 3.62-3.50 (m, 10H), 3.34 (s, 3H), 3.34-3.26 (m, 2H), 3.20-3.14 (m, 2H), 2.27 (t, J = 7.5 Hz, 2H), 1.62-1.54 (m, 2H), 1.48-1.38 (m, 2H), 1.41 (s, 9H), 1.31-1.14 (m, 12H). $^{13}$C-NMR (100 MHz, CDCl$_3$): $\delta$/ ppm = 179.4 (s), 178.8 (s), 79.3 (s), 71.8 (t), 70.5 (t), 70.4 (t), 70.3 (t), 69.5 (t), 58.9 (q), 47.9 (t), 46.6 (t), 34.2 (t), 29.4 (t), 29.3 (t, 3C), 29.1 (t), 29.0 (t), 28.4 (q, 3C), 26.7 (t), 24.8 (t).

Synthesis of benzyl-11-[tert-butoxycarbonyl-[2-[2-(2-methoxyethoxy)ethoxy]ethyl]amino]undecanoate (5): $^1$H-NMR (400 MHz, CDCl$_3$): $\delta$/ ppm = 7.33-7.27 (m, 5H), 5.07 (s, 2H), 3.64-3.49 (m, 10H), 3.34 (s, 3H), 3.37-3.27 (m, 2H), 3.22-3.16 (m, 2H), 2.31 (t, J = 7.5 Hz, 2H), 1.63-1.56 (m, 2H), 1.41 (s, 9H), 1.50-1.39 (m, 2H), 1.29-1.19 (m, 12H). $^{13}$C-NMR (100 MHz, CDCl$_3$): $\delta$/ ppm = 173.7 (s), 155.5 (s), 136.0 (s), 128.4 (d, 2C), 128.0 (d, 3C), 79.0 (s), 71.8 (t), 70.5 (t), 70.4 (t, 2C), 70.2 (t), 69.5 (t) 65.9 (t), 58.9 (q), 48.2 (t), 46.6 (t), 34.3 (t), 29.4 (t), 29.2 (t, 2C), 29.1 (t), 29.0 (t), 28.3 (q, 3C), 26.7 (t), 24.9 (t). HR-MS (+ESI): m/z calculated for C$_{30}$H$_{51}$NNaO$_7$ ([M + Na]$^+$): 560.3563. Found: 560.3559.
Synthesis of benzyl 11-[2-[2-(2-methoxyethoxy)ethoxy]ethylamino]undecanoate (6): $^1$H-NMR (400 MHz, CDCl$_3$): δ / ppm = 7.35-7.28 (m, 5H), 5.10 (s, 2H), 3.64-3.52 (m, 10H), 3.37 (s, 2H), 2.76 (t, J = 5.2 Hz, 2H), 2.58 (t, J = 7.3 Hz, 2H), 2.33 (t, J = 7.5 Hz, 2H), 1.71 (br, 1H), 1.65-1.58 (m, 2H), 1.50-1.42 (m, 2H), 1.33-1.15 (m, 12H). $^{13}$C-NMR (100 MHz, CDCl$_3$): δ / ppm = 173.6 (s), 136.1 (s), 128.5 (d, 2C), 128.1 (d, 3C), 71.9 (t), 70.6 (t), 70.5 (t), 70.4 (t), 70.2 (t), 66.0 (t), 59.0 (q), 50.0 (t), 49.3 (t), 34.3 (t), 30.1 (t), 29.5 (t), 29.4 (t), 29.3 (t), 29.2 (t), 29.0 (t), 27.3 (t), 24.9 (t).

Synthesis of benzyl 11-[(2-bromoacetyl)-[2-[2-(2-methoxyethoxy)ethoxy]ethyl]amino]-undecanoate (7): $^1$H-NMR (400 MHz, CDCl$_3$): δ / ppm = 7.37-7.29 (m, 5H), 5.09 (s, 2H), 3.98 (s, 1H), 3.84 (s, 1H), 3.62-3.58 (m, 8H), 3.58-3.58 (m, 3H), 3.362 + 3.359 (2 x s, 3H), 2.34 + 2.33 (t, J = 7.5 Hz, 2H), 1.66-1.47 (m, 4H), 1.32-1.18 (m, 12H). $^{13}$C-NMR (100 MHz, CDCl$_3$): δ / ppm = 173.59 (s), 173.55 (s), 176.3 (s), 166.5 (s), 136.0 (s, 2C), 128.4 (d, 4C), 128.1 (d, 6C), 71.84 (t), 71.81 (t), 70.74 (t), 70.53 (t, 2C), 70.45 (t), 70.43 (t), 70.38 (t), 68.78 (t), 68.75 (t), 66.0 (t, 2C), 59.0 (q, 2C), 50.1 (t), 48.4 (t), 46.26 (t), 46.20 (t), 34.2 (t, 2C), 29.4 (t, 2C), 29.24 (t, 2C), 29.11 (t, 2C), 29.00 (t), 28.99 (t), 27.3 (t), 27.1 (t), 26.70 (t, BrCH$_2$), 26.67 (t, BrCH$_2$), 26.3 (t), 24.84 (t), 24.82 (t).


10.71 (br, 1H), 7.45-7.41 (m, 2H), 7.34-7.23 (m, 23H), 7.10 (t, J = 13.1 Hz, 2H), 6.49 (s, 1H), 5.07 (s, 8H), 5.01 (s, 2H), 4.15-4.08 (m, 4H), 3.72-3.58 (m, 8H), 3.65-3.51 (m, 24H), 3.31 (s, 6H), 3.29 (s, 6H), 3.34-3.23 (m, 8H), 3.12-3.03 (m, 4H), 2.30 (t, J = 7.8 Hz, 8H), 1.63-1.55 (m, 8H), 1.52-1.42 (m, 8H), 1.28-1.16 (m, 48H). $^{13}$C-NMR (125 MHz, CDCl$_3$): $\delta$/ppm = 173.4 (s, 4C), 170.42 (s, 1C), 170.34 (s, 1C), 170.25 (s, 1C), 170.21 (s, 1C), 164.9 (s, 1C), 159.7 (s, 2C), 136.0 (s, 4C), 135.7 (s, 1C), 133.8 (s, 1C), 129.0 (d, 2C), 128.3+128.23+128.20+127.9 (d, 23C), 105.9 (d, 1C), 105.6 (d, 2C), 77.9 (t, 1C), 71.75 (t, 2C), 71.72 (t, 2C), 70.5+70.37+70.34+70.18 (t, 12C), 69.24 (t, 2C), 69.08 (t, 2C), 67.05 (t, 2C), 65.88 (t, 4C), 58.83 (q, 2C), 58.77 (q, 2C), 56.3 (t, 1C), 56.2 (t, 1C), 55.72 (t, 1C), 55.70 (t, 1C), 53.3 (t, 2C), 48.4 (t, 2C), 46.7 (t, 2C), 46.3 (t, 2C), 45.6 (t, 2C), 34.1 (t, 4C), 29.41+29.37+29.27+29.21+29.06+28.94 (t, 20C), 28.76 (t, 2C), 27.51 (t, 2C), 26.93 (t, 2C), 26.73 (t, 2C), 24.8 (t, 4C). HR-MS (+ESI): m/z calculated for C$_{126}$H$_{195}$N$_7$NaO$_{28}$ ([M + Na]$^+$): 2278.3975. Found: 2278.3966. HR-MS (+ESI): m/z calculated for C$_{126}$H$_{196}$N$_7$O$_{28}$ ([M + H]$^+$): 2256.4156. Found: 2256.4147. HR-MS (+ESI): m/z calculated for C$_{126}$H$_{195}$N$_7$Na$_2$O$_{28}$ ([M + 2Na]$^{2+}$): 1150.6934. Found: 1150.6896.


$^1$H-NMR (400 MHz, CDCl$_3$/DMSO-d$_6$): $\delta$/ppm = 8.9 (br s, 1H), 6.96 (br s, 2H), 6.36 (br s, 1H), 4.00-3.80 (m, 4H), 3.65-3.25 (m, 48H), 3.25-3.05 (m, 12H+8H), 3.05-2.85 (m, 4H), 2.10 (t, J = 7.4 Hz, 8H), 1.49-1.29 (m, 8H+8H), 1.28-0.95 (m, 48H). $^{13}$C-NMR (100 MHz, CDCl$_3$/DMSO-d$_6$): $\delta$/ppm = 176.4 (s, 4C), 170.55 (s, 1C), 170.48 (s, 1C), 170.40 (s, 1C), 170.30 (s, 1C), 164.3 (s, 1C), 159.8 (s, 2C), 133.8 (s, 1C), 105.9 (d, 1C), 105.6 (d, 2C), 71.4 (t, 4C), 70.4+70.1+70.34+70.05+69.9 (t, 12C), 68.9 (t, 2C), 68.7 (t, 2C), 66.6 (t, 2C),
58.52 (q, 2C), 58.49 (q, 2C), 56.1 (t, 1C), 55.9 (t, 1C), 55.44 (t, 1C), 55.36 (t, 1C), 52.9 (t, 2C), 48.1 (t, 2C), 46.5 (t, 2C), 46.0 (t, 2C), 45.4 (t, 2C), 33.8 (t, 4C), 29.9+29.0+28.9+28.6+28.4 (t, 2C), 28.4 (t, 2C), 27.1 (t, 2C), 26.6 (t, 2C), 26.4 (t, 2C), 24.5 (t, 4C). **HR-MS (+ESI):** m/z calculated for C$_{91}$H$_{167}$N$_7$O$_{28}$ ([M + 2H]$^{2+}$): 903.5940. Found: 903.5912. **HR-MS (+ESI):** m/z calculated for C$_{91}$H$_{166}$N$_7$NaO$_{28}$ ([M + H + Na]$^{2+}$): 914.5850. Found: 914.5797.

b) Materials: The solvents (CHCl$_3$, DMF, Et$_2$O, ethyl acetate, hexane, methanol, THF) were purchased in technical quality, purified by distillation, and dried according to standard procedures$^1$ if mentioned as dry solvent. 11-Aminoundecanoic acid (Fluka, 98%), benzyl bromide (Acros, 98%), bromoacetyl bromide (Fluka, 98%), Di-tert-butyldicarbonate (ABCR, 97%), DMEM and DMEM/FCS (Sigma-Aldrich), HCl aq (p.a.), Et$_3$N (Acros, 99%), NaCl (Grüssing, 99.5%), NaH (Adrich, 60% in mineral oil), NaHCO$_3$ (Fluka, 99.5%), Na$_2$SO$_4$ (Fluka, 99%), tetrabutylammonium iodide (Fluka, 99%), trifluoroacetic acid (Acros, 99%), Pd/C (Fluka, 10% Pd), tPr$_2$NEt (ABCR, 99%).

The preparation of compound 8 is described in reference$^2$.

2. Spectroscopic results

a) An infrared spectrum of the oleic acid protected iron oxide nanoparticles is shown in Figure 3-SI. The IR vibrations of the iron oxide nanocrystals with oleic acid as well as those of the nanoparticles functionalized with the ligands L-1 to L-5 are presented in Table SI-1.
Figure 3-SI. Infrared spectrum of the oleic acid capped Fe₃O₄ nanoparticles (NP-OA).

It cannot be concluded without any ambiguity if the double bond in oleic acid is stable under the experimental conditions. Considering the high temperatures during the growth process saturation of the molecule cannot be fully excluded. A similar discussion was given by Willis et al. for maghemite particles, which were synthesized from iron pentacarbonyl and oleic acid at 350 °C.³

Table SI-1. IR vibrations of the iron oxide nanocrystals.⁴⁻¹⁰

<table>
<thead>
<tr>
<th>sample</th>
<th>wave number [cm⁻¹]</th>
<th>explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>NP-OA</td>
<td>3004</td>
<td>ν_{vinyl} C-H (free oleic acid)</td>
</tr>
<tr>
<td></td>
<td>2954</td>
<td>ν_{as} CH₃</td>
</tr>
<tr>
<td></td>
<td>2925</td>
<td>ν_{s} CH₂</td>
</tr>
<tr>
<td></td>
<td>2855</td>
<td>ν_{s} CH₂</td>
</tr>
<tr>
<td></td>
<td>1711</td>
<td>ν C-O (free oleic acid)</td>
</tr>
<tr>
<td></td>
<td>1466</td>
<td>δ_{s} C-H</td>
</tr>
<tr>
<td></td>
<td>1377</td>
<td>δ_{s} CH₃</td>
</tr>
<tr>
<td></td>
<td>966</td>
<td>δ_{oop} C=C (free oleic acid)</td>
</tr>
</tbody>
</table>
b) The time dependence of the absorption spectra of sample NP-3 in 0.05 M HCl solution is shown in Figure 4-SI.
**Figure 4-SI.** The time dependence of the measured absorption spectra is shown for NP-3 in 0.05 M HCl solution. A lamp change in the spectrometer at 380 nm is causing a step in all spectra.

c) The Raman spectrum of the oleic acid protected iron oxide nanoparticles is compared to the spectra of sample NP-3 and magnetite (bulk) in **Figure 5-SI**.
Figure 5-SI. Raman spectra of the oleic acid capped iron oxide particles (NP-OA) and of iron oxide particles after a ligand exchange reaction with ligand L-3 (see Figure 1, NP-3). A Raman spectrum of pure bulk magnetite (Fe$_3$O$_4$) is included for a comparison. The dominant features are assigned to the spectra as described in ref. 64 in the article for bulk magnetite.

References